

Research Article

Assessment of Redox Status in Commercial Bottled Mineral Water

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ABSTRACT

Redox status in commercial bottled mineral water, ILOHAS, Mt. Fuji, Choju, Crystal Geysler, Volvic, Evian, Vittel, Perrier and Contrexville was studied. Among the 9 water, Choju and Perrier showed significantly larger DMPO-OH decay rate, k (min^{-1}). Next, redox related correlation was studied with 9 water samples. A strong correlation between the hardness of water and the amount of residue after lyophilization of each water sample was demonstrated. Significant correlations were observed between X-ray-induced $\bullet\text{OH}$ generation and pH of the water sample. Another significant correlation was also observed between free radical-reducing ability and 1 minus the ratio of hardness/residue content (1-H/R value) of the sample water, which is a value indicating a ratio of mineral contents other than (Ca) and magnesium (Mg) in the water sample. No notable correlations were recognized among other factors, such as correlation between DMPO-OH generation $C_{\text{DMPO-OH}}$ and residue weight, correlation between DMPO-OH decay rate and pH, and correlation between DMPO-OH decay rate and residue weight.

Keywords: Mineral water, Water hardness, Redox status, Electron paramagnetic resonance spin trapping, Electron paramagnetic resonance spin probing

Introduction

The quality of drinking water is important for our health. The hardness and/or mineral contents of drinking water are one of the factors used to evaluate water quality. Correlations between the hardness of drinking water and cardiovascular disease have become a concern [1-4]. Colloquial evidence also suggests that the quality of drinking water may have an anti-aging effect. Some essential trace elements in the mineral content, such as selenium, iron, copper, etc., can work as modifiers of the biological redox environment. The effective level of such bio-trace elements can be introduced and accumulated via daily water intake, although no strong direct redox activities are expected with drinking water. A great variety of brands of mineral water products are distributed by commercial companies, and they are widely available to purchase and consume. However, there is insufficient information available for healthy water choice on a scientific basis.

Spin-trapping techniques based on electron paramagnetic resonance (EPR) spectroscopy are to detect unstable free radical species [5]. A spin-trapping agent can react with a free radical species to give a corresponding spin adduct, which is a relatively stable free radical form, and become measurable using an EPR spectrometer. Each free radical species can be identified using the characteristic EPR spectra of the spin adduct. The spin adduct is not completely stable, and the EPR signal decays gradually by reduction and/or self-decomposition of the spin adduct. This instability of spin adducts has made the quantification of free radical species difficult in most experimental situations.

An applied method with EPR spin-trapping, which can simultaneously assess dual factors, i.e. X-ray-induced hydroxyl radical ($\bullet\text{OH}$) generation and free radical-reducing ability, has been reported [6,7]. In this method, the reaction mixture containing a spin-trapping agent only or with a subjected compound in an aqueous media, was irradiated with X-rays, then the time course of spin-adduct decay was measured using EPR spectroscopy. The reduction of the spin adduct during and after X-ray irradiation was corrected by extrapolation and a repetitive calculation based on the decay curve of the spin-adduct, thereby making it possible to accurately quantify $\bullet\text{OH}$ generation. In addition, the reduction rate of the spin adduct was proactively utilized to evaluate the free radical-reducing ability in the experimental environment. This simple chemical method can, therefore, be utilized to evaluate relatively weak redox activity in an aqueous sample.

In this paper, conventionally calculated hardness, actual lyophilized residue content, pH, X-ray-induced $\bullet\text{OH}$ generation ($C_{0\text{int}}$), and free radical-reducing ability (k), were measured and compared among some commercially available bottled mineral water products. The basic redox atmosphere of mineral water products as an index of water quality is discussed.

Materials and Methods

Chemicals

5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) was purchased from LABOTEC Co. (Tokyo, Japan). Other chemicals were of analytical grade.

Water samples

Deionized water, prepared using a Milli-Q system (Milli-Q water), was used as the control. The same milli-Q water was

also used to prepare phosphate buffer (PB) and boric acid buffer (BAB). Bottled mineral water product Choju-no-shima-no-mizu (Choju) was a gift from Amami-Oshima Nishikawa Shuzo Co., Ltd. (Tokunoshima, Japan). Other sample mineral water products were purchased commercially. Perrier, which is a soda water product, was used for experiments after degassing by strenuous stirring. Available information on the water products is summarized in Table 1.

pH measurement

Values of pH for the water samples were measured by three expert operators using different pH meters, and then averaged.

Weight of lyophilized residues

An aliquot (20 mL) of each water sample was placed into a plastic beaker. The water sample was frozen at -30°C and then lyophilized. The residue was weighed precisely using a 0.1 mg order scale.

X-ray-induced $\bullet\text{OH}$ generation and free radical-reducing ability

X-ray-induced $\bullet\text{OH}$ generation and free radical-reducing ability in water samples were estimated using previously reported methods [6]. A 15 mM DMPO water solution was prepared by adding 1.7 μL of DMPO to 1 mL of each water sample. The DMPO solution was irradiated by 32 Gy X-rays to generate DMPO-OH, which is an $\bullet\text{OH}$ adduct of DMPO. The details of X-ray irradiation are described below. A time course of EPR spectra of DMPO-OH for 10 min was measured using an X-band EPR spectrometer immediately after X-ray irradiation. The details of the EPR conditions are described below. The natural decay rate (k) of DMPO-OH was estimated from the decay curve of DMPO-OH after irradiation. The DMPO-OH decay constant k was used as a grade of free radical-reducing ability.

The $C_{0\text{exp}}$ of DMPO-OH was obtained by extrapolating the experimental decay curve of DMPO-OH to time = 0 (end of irradiation). $C_{0\text{exp}}$ is a result of linear DMPO-OH generation and simultaneous decay of DMPO-OH during X-ray irradiation. When the decay of DMPO-OH during X-ray irradiation could be eliminated, the intact total amount of DMPO-OH generation during X-ray irradiation, i.e. $C_{0\text{int}}$, can be obtained. Therefore, $C_{0\text{int}}$ was predicted using the following calculation. At first, $C_{0\text{exp}}$ was used as the initial prediction of $C_{0\text{int}}$. Then the generation of DMPO-OH during X-ray irradiation was simulated while considering the decay of DMPO-OH with the decay rate of k to obtain a prediction of the net generation of DMPO-OH ($C_{0\text{net}}$). By incrementing the $C_{0\text{int}}$ prediction value until $C_{0\text{net}}$ becomes equal to $C_{0\text{exp}}$, the final decision of $C_{0\text{int}}$, i.e. the intact amount of DMPO-OH generation during X-ray irradiation, was obtained.

X-ray irradiation

X-ray irradiation was performed at PANTAK 320S (Shimadzu, Kyoto, Japan). Effective energy was 80 keV using the following conditions: the X-ray tube voltage was 200 kV, X-ray tube current 20 mA, and the thickness and materials of the pre-filter 0.5 mm copper and 0.5 mm aluminum. The dose rate of X-ray irradiation was 3.2 Gy/min when the distance between the X-ray tube and the sample was 300 mm.

X-band EPR measurement

One hundred microliters of the X-ray-irradiated DMPO solution was drawn up into PTFE tubing (i.d. 0.32 ± 0.001 inches, wall 0.002

Table 1: Descriptive Mineral Contents and Experimental Values of Mineral Water Products.

Water Product	pH	Na (mg/L)	Mg (mg/L)	Ca (mg/L)	K (mg/L)	V (mg/L)	Hardness ^a (mg/mL)	Residual (mg/L)
Milli-Q water	6.5 ± 0.98	---	---	---	---	---	0 ^b	32 ± 59
I LOHAS ^c	7.8 ± 0.04	8.6	3	10	1.3	---	32	131 ± 23
Mt. Fuji ^d	7.9 ± 0.07	10	5	16	1.9	0.068	63	143 ± 46
Choju ^e	7.8 ± 0.53	19.4	18.6	50.5	2.3	---	198	269 ± 45
Crystal Geyser	7.4 ± 0.19	11.3	5.4	6.4	1.8	0.055	38	89 ± 9
Volvic	7.5 ± 0.08	11.6	8	11.5	6.2	---	61	150 ± 28
Evian	7.7 ± 0.44	7	26	80	0	---	304	389 ± 8
Vittel	7.8 ± 0.36	7.7	20	94	5	---	315	379 ± 52
Perrier	5.9 ± 0.15	11.8	6.8	155	1.3	---	415	523 ± 8
Contrexville ^f	7.5 ± 0.23	9.4	74.5	468	2.8	---	1463	2666 ± 29

^a Hardness (mg/L) = Ca contents (mg/L) × 2.5 + Mg contents (mg/L) × 4.1

^b Hardness value of Milli-Q water was set as zero for descriptive purposes.

^c I LOHAS, Coca-Cola East Japan Co., Ltd. (Tokyo, Japan).

^d Fujisanroku-no-oishii-ten-nen-sui, Pokka Sapporo Food & Beverage Ltd. (Nagoya, Japan).

^e Choju-no-sima-no-mizu, Amamiohshima Nishikawa Shuzo Co., Ltd. (Tokunoshima, Japan).

^f Contains 1121 mg/L of sulfate.

“---” indicates “no data”

± 0.0005 inches; ZEUS, Orangeburg, SC), placed in the TE mode cavity using a special sample holder, and measured using an X-band EPR spectrometer JES-TE100 (JEOL, Tokyo Japan). The second peak from the lower field of 4 lines of DMPO-OH was recorded using the following conditions: microwave frequency 9.45 GHz, microwave power 2 mW, lower magnetic field 336.1 mT, field sweep width 1.25 mT, field sweep resolution 1024 points, sweep time 15 s, time constant 0.03 s, field modulation frequency 100 kHz, and field modulation width 0.063 mT. The EPR measurements were repeated 10 times at 1 min intervals for each sample. EPR data acquisition was controlled by a WIN-RAD ESR Data Analyzer System (Radical Research, Inc., Hino, Tokyo). The acquired EPR spectra were analyzed using an in-house line-fitting program. A Gaussian line shape was fitted onto the acquired EPR spectra, then the signal height and line width of the fitted Gaussian line was measured. The EPR signal intensity was calculated as (signal height) × (line width).

Statistical analysis

Statistical differences were estimated using Scheffe’s F test. Grades of significance were estimated by $p < 0.05$ or $p < 0.01$.

Results

Figure 1A shows a comparison of X-ray-induced DMPO-OH generation, C_{0int} (μmol/L), i.e. X-ray-induced •OH generation, among the several water samples. Perrier showed significantly lower X-ray-induced DMPO-OH generation. Because the DMPO-OH generation C_{0int} value obtained in this paper was a corrected value considering DMPO-OH decay during X-ray irradiation, the lower C_{0int} indicates the difficulty of •OH generation in the sample. One possible factor to reduce C_{0int} was •OH cancelation/trapping by residual constituents, although milli-Q water (residual content was 32 ± 59 mg/L) and 100 mM buffers (residual contents should be 13.9–14.1 g/L for PB and for 5.2–5.4 g/L BAB) showed almost the same C_{0int} values. This fact suggested that the direct cancelation of •OH by inorganic residual constituents was sufficiently small. Therefore, DMPO-OH generation can simply indicate •OH generation in this reaction system.

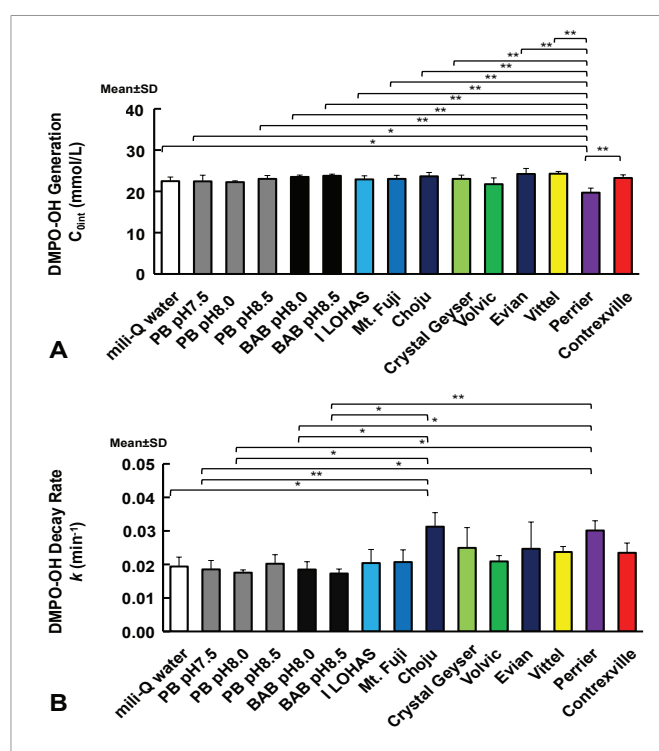


Figure 1: X-ray-induced •OH generation and free radical-reducing ability in water samples. (A) X-ray-induced DMPO-OH generation, C_{0int} (μmol/L) indicates •OH generation, in the reaction mixture prepared using each water sample. The reaction mixture, containing 15 mM DMPO, was irradiated with 32 Gy of X-rays. (B) Decay rate constant of DMPO-OH, k (min⁻¹), indicates the free radical-reducing ability in the water sample. Columns and error bars indicates average ± SD of several independent experiments. The numbers of experiments were at least 4. * and ** indicate significance as $p < 0.05$ and $p < 0.01$, respectively. Number of experiments is indicated on each column.

Figure 1B shows a comparison of DMPO-OH k (min⁻¹), i.e. stable free radical-reducing ability, among several water samples. The decay rate of DMPO-OH was significantly faster in Choju and Perrier compared with the control water samples, i.e. milli-Q, PB, and

BAB. A faster k value indicated a reductive atmosphere. A similar or much stronger reductive condition can be created by the addition of reductive antioxidants, such as ascorbic acid, berberine, glutathione, etc [6,7].

Table 1 summarizes the pH, descriptive mineral contents, hardness values, and experimentally obtained residual content of the water samples. Measuring the pH of the water samples, which had no buffer effect, was very unstable, and error values were relatively large. Perrier showed significantly lower pH, which was probably due to remaining CO_2 . Other water samples showed slight alkaline pH values. The hardness, which indicated total permanent water hardness expressed as equivalent of CaCO_3 , can be calculated based on the contents of calcium (Ca) and magnesium (Mg) ions. Because the correlation between the hardness and residue content showed a strong linear relationship (Figure 2), the main mineral contents were almost fully due to Ca and Mg salts.

Correlations among several parameters obtained in this study were analyzed to find a possible determinant factor for water quality. First, the factors that affected DMPO-OH generations were examined (Figure 3). No correlation was observed between DMPO-OH generation and the DMPO-OH decay rate (Figure 3A). In other words, $\bullet\text{OH}$ cancelation and free radical reduction were independent factors of each other. Therefore, it is important that these two anti-oxidative factors must be regulated separately. In addition, no correlation was observed between DMPO-OH generation and the residual content (Figure 3B). Again, the findings suggested that the direct $\bullet\text{OH}$ cancelation by residual constituents, i.e. inorganic contents, was small. A weak correlation was observed between DMPO-OH generation, i.e. X-ray-induced $\bullet\text{OH}$ generation, and the pH of the water samples (Figure 3C).

The factors that affected the DMPO-OH decay rate were tested. The DMPO-OH decay rate showed no correlation with pH (Figure 4A), nor the residue content (Figure 4B). An index value, 1-H/R, which was calculated as 1 minus the ratio of hardness/residue content, was proposed here. The 1-H/R is indicating a ratio of mineral contents other than Ca and Mg. Figure 4C shows the relationship between free radical-reduction ability and 1-H/R values of the water samples. There was a weak correlation between free radical-reducing ability in the water samples and 1-H/R value. This suggested that some unknown trace minerals, other than Ca and Mg, probably affect the redox atmospheres of the mineral water samples. No correlation ($R^2 = 0.0124$) between 1-H/R and DMPO-OH generation was observed (data not shown).

Discussion

It is known that components of each natural water are different, and the calculation of hardness is determined as $\text{Ca (mg/L)} \times 2.5 + \text{Mg (mg/L)} \times 4.1$ [8,9]. The degree of water hardness was determined, as shown as in Table 2.

The milli-Q water indicated a residual value in this experiment (Table 1); however it should be zero for milli-Q water. This result is probably due to contamination of dust and/or scaling error. The individual values for 3 milli-Q samples measured were -0.11, 0.04, and 2.02 mg/20 mL.

DMPO-OH generation and DMPO-OH decay are completely different factors. The DMPO-OH generation is a simply observing the

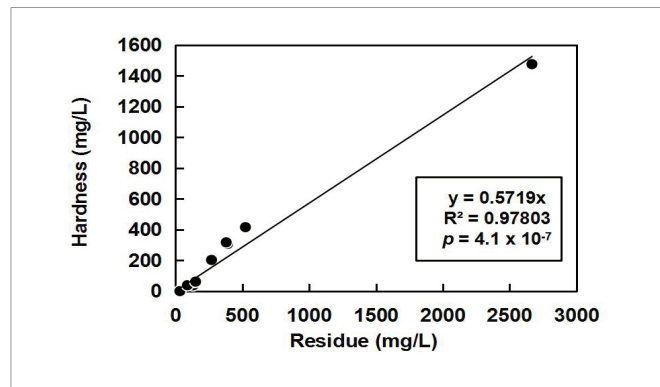


Figure 2: Correlation between hardness and residue level. The hardness and residues values indicated in Table 1 were plotted. A strong linear approximation was obtained.

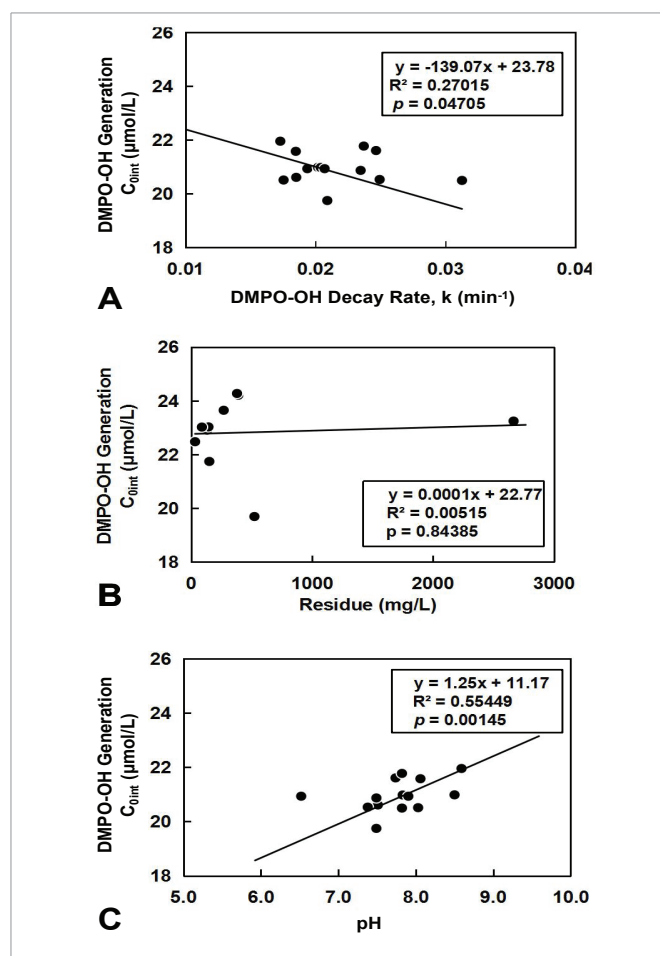


Figure 3: Correlations of DMPO-OH generation C_{0int} and specific parameters. (A) Correlation between DMPO-OH generation and DMPO-OH decay rate. (B) Correlation between DMPO-OH generation C_{0int} and residue weight. (C) Correlation between DMPO-OH generation C_{0int} and pH. Significance was estimated by $p < 0.05$. A weak correlation was obtained between DMPO-OH generation and pH.

hydroxyl radical ($\bullet\text{OH}$) generated in the sample solution. The $\bullet\text{OH}$ could be immediately trapped by DMPO, in other word, $\bullet\text{OH}$ can oxidize DMPO, and give DMPO-OH as a product. Since a correlation was observed between DMPO-OH generation and the pH (Figure 3C), a correlation between pH and $\bullet\text{OH}$ generation was predicted.

However, a much precisely planned experiment may be required to confirm the relation between X-ray induced $\bullet\text{OH}$ generation and pH in the sample water. The DMPO-OH is a relatively stable free radical species, which has a nitroxyl radical on it. The DMPO-OH decay is monitoring reduction of nitroxyl radical in the DMPO-OH molecule. The $\bullet\text{OH}$ generation, which may be depending on pH, was larger in Perrier, however the DMPO-OH decay, which may be depending on contents of reductive mineral, was larger both in Choju and Perrier.

Besides the taste, it will be of interest to determine the antioxidant

capacity of the water samples. Evidence of additional nutrient water has been widely discussed [10]. However, the function of commercially available drinking water has been rarely reported or discussed. Mineral-rich water may provide an important supplementary contribution to total Ca and Mg intake [11]. In this study nine natural water samples, which are available commercially were examined to determine their antioxidant capacity, which was determined using EPR spectroscopy [6].

Water quality is generally evaluated by examining pollutants, chemical contaminants, and/or bacteria, but there is no method to evaluate water functionality. In other words, most assay methods excel at evaluating negative indexes and/or demerits of the subject, but they are poor at evaluating the beneficial features of a subject. The free radical-reducing ability, which is a quantitative factor for the anti-oxidative function, can be positive index of water quality. Water contains various minerals and other components, some of which even stimulate cellular transduction signals. Wang et al. [12] reported that the components including organic extracts, activate Nrf2 and binds to ARE, activating various antioxidative stress relating enzymes. Main contents of inorganic minerals, i.e. Ca and Mg, are redox inactive and have no recognizable redox functionality. Such functionless minerals may be no problem on the quality mineral water. However, some miner minerals, such as selenium, iron, copper, and etc., are redox active and they could give a redox behavior of the water. Therefore, we proposed an index such as 1-H/R, an indicator of mineral contents other than Ca and Mg, to set evaluation standards for functions of drink water.

Regarding sulfurous components in the mineral water, Benedetti et al. reported that drinking 500 ml/day mineral water for 2 weeks, which contained 7.25 mg/500 ml of sulfurous components, in 40 subjects (18 men and 22 women, ages 41–55 years). The drinking water ameliorated oxidative stress in blood plasma, which was examined using oxidative stress biomarkers such as hydroperoxides, malondialdehyde, carbonyls, and advanced oxidation protein products [13]. They also found that tocopherols, carotenoids, and retinol remained almost unchanged before and after treatment in both groups.

Deep-sea water (DSW) has also been reported to contain high levels of minerals, such as sodium (Na), potassium (K), Ca, and Mg, compared with surface- and middle-sea water [14-16]. DSW is characterized by high purity, low temperature, and high nutrients and minerals, and has recently been tested as a multifunctional materials in the food, agricultural, cosmetic, and medical fields. DSW exhibits hardness (ppm) of 310.05, 890.80, and 517.96 ppm for DSW collected at 300, 900, and 1,500 m, respectively. Hardness of soft and very hard water is less than 7.0 and over 170 ppm, respectively, as shown in Table 2. The hardness of Choju and Perrier, for which the DMPO-OH decay rate was significant larger, 269 ± 45 and 523 ± 8 mg/L, respectively (Table 1). It was noted that Evian, Vittel, and Contrexville showed greater hardness but did not show a significantly larger decay rate. Hardness might be a factor to determine the antioxidant effect, however, other factors should not be eliminated. It was reported that DSW (hardness: 1000 ppm) showed an amelioration of body-weight increase, blood sugar, and serum lipids in ob/ob (obese) mice [14,17]. Recently, the health effects of DSW from sea water below 700 m in the outer sea of Hua-Lien County, Taiwan, have been demonstrated [14,18]. This DSW was reported as having beneficial effects on hepatic lipid accumulation [14]. It was also reported that electro dialysis (ED)-

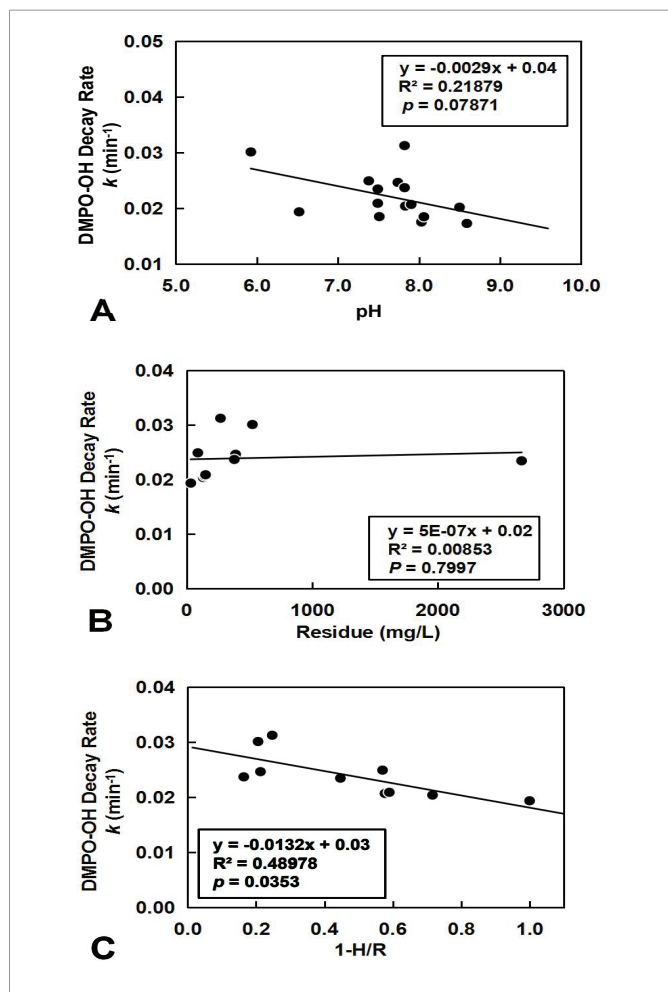


Figure 4: Correlations of DMPO-OH decay rate k and specific parameters. (A) Correlation between DMPO-OH decay rate and pH. (B) Correlation between DMPO-OH decay rate and residue weight. (C) Correlation between DMPO-OH decay rate and 1-H/R value. Significance was estimated by $p < 0.05$. A weak correlation was obtained between 1-H/R value and DMPO-OH reduction rate k .

Table 2:

Degree of Hardness	ppm (or mg/L)
Soft	<17.0
Slightly Hard	17.1–60
Moderately Hard	60–120
Hard	120–180
Very Hard	>180

<https://www.wqa.org/learn-about-water/perceptible-issues/scale-deposits>

treated DSW (ED DSW; hardness: 4685.90 ppm) could decrease serum lipids and improve the blood cholesterol profile [14,18]. DSW drinking waters could upregulate the expression of hepatic low-density-lipoprotein receptor and cholesterol-7 α -hydroxylase genes to reduce serum cholesterol levels, and concurrently decrease serum lipid oxidative levels [14,19]. Miyamura et al. [18] also discussed the role of DSW in prevention of atherosclerosis. It was noted that the DSW Choju showed a strong free radical reducing ability in this study.

Conclusion

Redox status in commercial bottled mineral water, ILOHAS, Mt. Fuji, Choju, Crystal Geyser, Volvic, Evian, Vittel, Perrier and Contrexville was studied using an applied EPR spin-trapping technique. A significant higher free radical reducing ability was observed in Choju and Perrier. Among the 9 bottled water, significant correlations were observed between the hardness of water and the amount of residue after lyophilization, between X-ray-induced \bullet OH generation and pH in the water samples, and between free radical-reducing ability and 1-H/R value in the water samples.

Conflict of Interest

The authors have no conflict of interests in this study.

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Author Contributions

K.M., Y.O, M.K., M.N. and I.N. contributed to the whole analyses and discussion of the results. H.P.I. and H.J.M. contributed to the experimental planning and discussion of the results. S.S., H.I., H.M. and T.O. contributed to all of these aspects in this study, including drafting the manuscript and executing the final revisions. All authors approved the final manuscript prior to publication.

References

1. Lindeman, RD., Assenzo, JR. (1964) Correlations between water hardness and cardiovascular deaths in Oklahoma counties. *Am J Public Health Nations Health*, 54(7): 1071-1077.
2. Dudley, EF., Beldin, RA., Johnson, BC. (1969) Climate, water hardness and coronary heart disease. *Journal of Chronic Diseases*, 22(1): 25-48.
3. Masironi, R. (1970) Cardiovascular mortality in relation to radioactivity and hardness of local water supplies in the USA. *Bull World Health Organ*, 43(5): 687-697.
4. Stocks, P. (1973) Mortality from cancer and cardiovascular diseases in the county boroughs of England and Wales classified according to the sources and hardness of their water supplies, 1958-1967. *J Hyg (Lond)*, 71(2): 237-252.
5. Davies, MJ. (2016) Detection and characterization of radicals using electron paramagnetic resonance (EPR) spin trapping and related methods. *Methods*, 109: 21-30.
6. Ueno, M., Nakanishi, I., Matsumoto, K. (2013) Method for assessing X-ray-induced hydroxyl radical-scavenging activity of biological compounds/materials. *J Clin Biochem Nutr*, 52(2): 95-100.
7. Matsumoto, C., Sekine-Suzuki, E., Nyui, M., Ueno, M., Nakanishi, I., Omiya, Y., et al. (2015) Analysis of the antioxidative function of the radioprotective Japanese traditional (Kampo) medicine, hangeshashinto, in an aqueous phase. *J Radiat Res*, 56(4): 669-677.
8. Miyamura, M., Yoshioka, S., Hamada, A., Takuma, D., Yokota, J., Kusunose, M., et al. (2004) Difference between deep seawater and surface seawater in the preventive effect of atherosclerosis. *Biol Pharm Bull*, 27(11): 1784-1787.
9. Water Hardness Calculator. <http://www.lenntech.com/ro/water-hardness.htm>.
10. Nutrients in Drinking Water - World Health Organization; www.who.int/water_sanitation_health/dwq/nutrientsindw.pdf
11. Galan, P., Arnaud, MJ., Czernichow, S., Delabroise, AM., Preziosi, P., Bertrais, S., et al. (2002) Contribution of mineral waters to dietary calcium and magnesium intake in a French adult population. *J Am Diet Assoc.*, 102(11): 1658-1662.
12. Wang, S., Zhang, H., Zheng, W., Wang, X., Andersen, ME., Pi, J., et al. (2013) Organic extract contaminants from drinking water activate Nrf2-mediated antioxidant response in a human cell line. *Environ Sci Technol*, 47(9): 4768-4777.
13. Benedetti, S., Benvenuti, F., Nappi, G., Fortunati, NA., Marino, L., Aureli, T., et al. (2009) Antioxidative effects of sulfurous mineral water: protection against lipid and protein oxidation. *European Journal of Clinical Nutrition*, 63(1): 106-112.
14. Chen, IS., Chang, YY., Hsu, CL., Lin, HW., Chang, MH., Chen, JW., et al. (2013) Alleviative effects of deep-seawater drinking water on hepatic lipid accumulation and oxidation induced by a high-fat diet. *Journal of Chinese Medical Association*, 76(2): 95-101.
15. Toyota, T., Nakashima, T. (1998) Comparison of the effects of water-soluble (EDTA) and particulate (chelex-100) synthetic ligands on the growth of phytoplankton population in the disphotic zone seawater. *Journal of Oceanography*, 54(1): 19-28.
16. Katsuda, S., Yasukawa, T., Nakagawa, K., Miyake, M., Yamasaki, M., Katahira, K., et al. (2008) Deep-sea water improves cardiovascular hemodynamics in Kurosawa and Kusanagi-Hypercholesterolemic (KHC) rabbits. *Biol Pharma Bull*, 31(1): 38-44.
17. Hwang, HS., Kim, HA., Lee, SH., Yun, JW. (2009) Anti-obesity and antidiabetic effects of deep sea water on ob/ob mice. *Mar Biotechnol (NY)*, 11(4): 531-539.
18. Chang, MH., Tzang, BS., Yang, TY., Yang, HC., Hsiao, YC., Chen, YC. (2011) Effects of deep-seawater on blood lipids and pressure in high-cholesterol dietary mice. *Journal of Food Biochemistry*, 35(1): 241-259.
19. Hsu, CL., Chang, YY., Chiu, CH., Yang, KT., Wang, Y., Fu, SG., et al. (2011) Cardiovascular protection of deep-seawater drinking water in high-fat/cholesterol fed hamsters. *Food Chem*, 127(3): 1146-1152.